Attorney Docket No. 5585-70293-01 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Boden et al
Serial No.: 10/521,628
Filed: September 8, 2005
Confirmation No: 2023
Group Art Unit: 1654
Examiner: HA, Julie

For: *BETA SHEET TAPES RIBBONS IN TISSUE ENGINEERING*

February 5, 2008

Commissioner for Patents
Post Office Box 1450
Alexandria, Virginia 22313-1450

DECLARATION UNDER 37 C.F.R § 1.132
OF AMALIA AGGELI, PhD.

Sir:

I, Amalia Aggeli, do hereby declare and say as follows:

1. I received my Doctor of Philosophy degree (PhD.) from The University of Leeds. I am currently, Deputy Director of the Centre for Self Organising Molecular Systems (SOMS) and Royal Society University Research Fellow in the School of Chemistry at the University of Leeds. A curriculum vitae is attached herewith at Tab A.

2. I am a co-inventor of the above-identified patent application (hereinafter referred to as "the '628 application"). I am also the first named author of Aggeli et al (Peptide Science, Present and Future, 1999, 30-33 (hereinafter referred to as "Aggeli et al").

3. My research interests are mainly focused upon the understanding of molecular self assembly and on using biologically-inspired self-assembly as a route to engineering novel functional nanostructured materials and devices. The centre of my investigations is the ß-sheet motif and the rational design of ß-sheet-forming peptides that give rise to

novel μm-long polymers: nanotapes, ribbons, fibrils, and fibres, which form nematic liquid crystalline solutions, gels and single-molecule thick surface coatings.

4. The Examiner questions the clarity of the term "net charge" in claim 1 of the '628 application. A net charge is quite simply the final charge of either an individual peptide or a pair of complimentary peptides. Claim 1 is restricted to peptides with a net charge of either +2 or -2 overall. Thus peptides of the P11-3 formula (which has a net charge of -2) and peptide P11-5 (which has a net charge of +2) are within the scope of the definition of claim 1, as are a pair of complimentary peptides that would produce an overall net charge of +2 or -2. Individual peptides falling outside of the scope of claim 1 are be P11-1 (net charge 0), P11-2 (net charge 0), P11-4 (net charge +3), P11-6 (net charge -4) and P10-7 (net charge +4). The calculation of charge of complimentary peptides obey the basic mathematical principles of arithmetic so an example of a complimentary pair of peptides within the scope of claim 1 is the pairing of P11-6 (net charge of -4) and P11-5 (net charge of +2) so the complimentary pair would have an overall net charge of -2. An example of a pair of complimentary peptides falling outside the scope of claim would be the paired P11-3 (net charge -2) and P11-4 (net charge +3) peptides giving an overall net charge of +1.

The net charge of paired peptides is merely the arithmetic calculation by addition of positive or negative integers.

5. Further research has been conducted, based upon the present invention and on the critical observation that a +2 or -2 net charge of individual or complimentary peptides when in solution at physiological pH is essential for the peptides to form a material that acts as a biological scaffold.

This can be evidenced by the following two studies:

I. A family of systematically designed amphiphilic peptides bearing a net positive or negative charge ranging from 0 to 6 have been produced and studied in physiological-like solution conditions (Tab B, Table 1, appended hereto). These peptides share the same primary structure apart from the systematic increase of the net peptide charge. FTIR spectroscopy was employed to quantify peptide aggregation as a function of peptide charge. It was discovered that peptides with net positive or negative charge equal to or less than 2 were able to self-assemble in physiological-like solution conditions and adopt predominantly a beta-sheet structure. However only peptides with a net +2 or -2 charge produced soluble beta-sheet aggregates, whilst peptides with less charge produced insoluble aggregates. Increasing the net peptide charge to 3 or 4 caused decreased % of beta-sheet structure present, implying decreased tendency for peptide self-assembly in solution. This was further corroborated by the fact that these solutions of peptides with the higher net charge were more fluid-like compared to the viscous, gel-like nature of the solutions containing peptides with -2 or +2 net charge. This thorough study demonstrates in a quantitative manner the precise role of peptide charge on: 1) self-assembly in physiological solution conditions; 2) solubility of the aggregates and gelation.

II. Based on the above data, a new family of amphiphilic peptides were then designed with a +2 or -2 net charge per peptide and systematically varying peptide primary structure. The purpose of these studies was to demonstrate the generality of the charge principle for the stabilisation of self-assembling peptide gels in biological-like solution conditions. In this family of peptides, the polar amino acid residues were systematically varied from glutamine, to asparagine, threonine and serine (Tab C Table 2 and Tab D Table 3). It was discovered that irrespective of their primary structure, all the peptides formed gels (Tab E Figure 1). The charge principle was further tested in the context of purely polar peptides, as opposed to all the above peptides which are amphiphilic. Surprisingly it was shown that completely polar peptides bearing

the right number of net charge (+2 or -2) were able to self-associate into gel networks in biological solutions (Tab F Table 4). Such completely polar peptides might be on average more biocompatible compared to their amphiphilic counterparts, based on reduced expected cell membrane toxicity.

6. Turning now to the disclosure of Aggeli et al, this document merely shows the structure of peptide P11-3 (Figure 1) not any function. Indeed, with reference to Figure 2 this document clearly shows that at pH 7 the peptide P11-3 is a **fluid**. What Aggeli et al teaches is that P11-3, at pH values less than 4, is gel but at higher pH values the gel converts to a clear liquid. "*At pH values less than 4, i.e. pH values lower than the pKa of the glutamate side chains, the peptide molecules self-assemble into stable structures and form gels.* ***In contrast,*** *at pH values higher than the pKa of the glutamate side chains, the electrostatic repulsive forces between negatively charged glutamate side-chains, disrupt the polymeric β-sheet structure, and convert the gel into a* ***clear fluid***".

Not only does Aggeli et al not assign a function to the P11-3 peptide but it is totally counterintuitive to the present claimed invention of the '628 application since the peptide of the present invention is required to be a gel not a fluid at physiological pH (i.e. at a pH of around 7.6) in order to form β-sheet structures of ribbons, tapes or fibrils that are capable of being used as biological scaffolds for tissue engineering.

The skilled man, taking the teachings of Aggeli et al would not select the P11-3 peptide as a candidate for forming β-sheet structures at physiological pH since Aggeli et al clearly demonstrate that it reverts to a fluid monomer from a gel at a pH value above 4, something which the present invention seeks to avoid.

7. In summary, Aggeli et al neither discloses the functioning capability of peptide P11-3 nor does it teach towards it being able to self-assemble into a β-sheet structure at physiological pH and salt concentrations. There is no motivation for the skilled man in

Aggeli et al to consider peptide P11-3 as a potential candidate for β-sheet formation at physiological pH and subsequent uses in tissue engineering. There is no motivation in Aggeli et al to look for self assembly β-sheet forming peptides at a pH above 4 since Aggeli teaches that they turn to a clear liquid. This behaviour was later on attributed to the presence of salts, as supported by further studies. The studies reported in Aggeli et al were conducted in pure water (no added salt), whilst the discoveries described in the current invention are only relevant to solutions of neutral pH in the presence of the biological relevant concentration of NaCl of ~130 mM. We have recently discovered much to our surprise that this added salt greatly affects peptide self-assembly in water and shifts the whole self-assembling curve to much higher pHs, effectively by partially screening in an extremely efficient way the peptide charges. Thus the same peptide at neutral pH in the absence of added salt produces a fluid clear solution of monomers, whilst at neutral pH in the presence of 130 mM added NaCl produces a self-supporting gel.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Amalia Aggeli, PhD.

5 February 2008
Date 5/2/2008

TAB A

A.Aggeli : RESEARCH

Publications

Peer-reviewed publications :

Aggeli, A, Hamodrakas, SJ, Komitopoulou, K, & Konsolaki, M., Tandemly repeating peptide motifs and their secondary structure in *C.capitata* eggshell proteins Cc36 and Cc38, *Int.J.Biol.Macrom.*,13, 307-315, 1991.

Aggeli, A, Boden, N., Cheng, Y.L., Findlay, JBC, Knowles, PF, Kovatchev, P. & Turnbull, P., Peptides modelled on the transmembrane region of the slow voltage-gated IsK potassium channel: Structural characterisation of peptide assemblies in the β-strand conformation, *Biochemistry*, 35, 16213-16221, 1996.

Aggeli, A, Bell, M, Boden, N, Keen, J, Knowles, PF, .McLeish, TCB, Pitkeathly, M & Radford, SE., Responsive gels formed by the spontaneous self-assembly of peptides into polymeric, β-sheet tapes, *Nature*, 386, 259-262, 1997.

Aggeli, A, Bell, M, Boden, N, Keen, JN, McLeish, TCB, Nyrkova, I, Radford, SE, & Semenov, A, Engineering of peptide-based β-sheet nanotapes, *Journal of Materials Chemistry, Special Issue on Molecular Assemblies and Nanochemistry*, 7, 1135-1145, 1997.

Aggeli, A, Bannister, M., Bell, M., Boden, N., Findlay, JBC, Hunter, M., Knowles, P.F. & Yang, JC, Conformation and ion-channelling activity of a 27-residue peptide modelled on the single transmembrane segment of the IsK (minK) protein, *Biochemistry*, 37, 8121-8131, 1998.

Benaki, DC, Chryssikos, GD, Yiannopoulos, YD, Brumley, E, Aggeli, A, Boden, N, Case, ST & Hamodrakas, SJ, Laser-Raman and FT-IR spectroscopic studies of peptide-analogues of silkmoth chorion protein segments, *Int.J.Biol.Macrom.*, 23, 49-59, 1998.

Nyrkova, IA, Semenov, AN, Aggeli, A & Boden, N, Fibril stability in solutions of twisted beta-sheet peptides : a new kind of micellisation in chiral systems, *Eur Phys J B*, 17, 481-497, 2000.

Nyrkova IA, Semenov AN, Aggeli A, Bell M, Boden N, McLeish TCB, Self-assembly and structure transformations in living polymers forming fibrils, *Eur Phys J B*, 17, 499-513, 2000

Aggeli, A., Boden, N., Fytas, G., McLeish, TCB, Mawer, P & Vlassopoulos, D., Structure and dynamics of self assembled peptide polymeric β-sheet tapes by dynamic light scattering, *Biomacromolecules*, 2, 378-388, 2001.

Aggeli, A., Nyrkova, I., Bell, M., Harding, R., Carrick, L., McLeish, TCB, Semenov, A. & Boden, N., Hierarchical self-assembly of chiral units as a model for β-sheet macromolecules which form gels and liquid crystals *Proc.Natl.Ac.Sci. USA,* 98, 11857-11862, 2001

Aggeli, A, Bell, M., Carrick, L, Fishwick, C, Harding, R. Mawer, P, Radford, S, Strong, A, Boden N, pH as a trigger of peptide beta-sheet self-assembly and reversible switching between nematic and isotropic phases *JACS,* 125, 9619-9628, 2003

Aggeli, A., Bell, M, Boden, N, Carrick, L, Strong, A, Self-assembling peptide polyelectrolyte beta-sheet complexes form nematic hydrogels *Angewandte Chemie Int. Ed.,* 42, 5603-5606, 2003

Fishwick, C, Beevers, A, Carrick, L, Whitehouse, C, Aggeli, A, Boden, N, Simulating the structures of helical beta- tapes and twisted ribbons : the role of side-chain interactions on twist and bend behaviour *Nano Letters,* 3, 1475-1479, 2003

Meegan, J, Aggeli, A, Boden, N, Brydson, R, Brown, A, Carrick, L, Brough, A, Hussain, A, Ansell, R, Designed self-assembled beta-sheet peptide fibrils as templates for silica nanotubes, *Advanced Functional Materials,* 14, 31-37, 2004

Kayser, V, Turton, D, Aggeli, A, Beevers, A, Reid, G, and Beddard, G, Fluorescence studies and tryptophan-tryptophan energy migration in pH-triggered self-assembled beta-sheet ribbons, *JACS,* 126, 336-343, 2004

Carrick, L, Tassieri, M, Waigh, TA, Aggeli, A, Boden, N, Bell, C, Fisher, J, Ingham, E, Evans, R, The internal dynamic modes of charged self-assembled peptide fibrils, *Langmuir*, vol.21, 3733-3737, 2005

Whitehouse, C, Fang, J, Aggeli, A, Bell, M, Brydson, R, Fishwick, C, Henderson, J, Knobler, CM, Owens, RW, Thomson, NH, Smith, DA & Boden, N, Adsorption and self-assembly of peptides on mica substrates, *Angewandte Chemie Int. Ed.*, vol. 44, 1965-1968 2005.

Bell CJ, Carrick LM, Katta J, Jin ZM, Ingham E, Aggeli A, Boden N, Waigh TA, Fisher J , Self-assembling peptides as injectable lubricants for osteoarthritis, Journal of Biomedical Materials Research Part A, vol. 78A, 236-246, 2006

Davies RPW, Aggeli A, Beevers AJ, Boden N, Carrick LM, Fishwick CWG, McLeish TCB, Nyrkova I, Semenov AN, Self-assembling beta-sheet tape forming peptides, *Supramolecular Chemistry*, vol. 18, 435-443, 2006

Firth, A, Aggeli, A, Burke, J.L, Yang, X & Kirkham, J., Biomimetic sef-assembling peptides as injectable scaffolds for hard tissue engineering, *Nanomedicine*, vol. 1, 189-199, 2006

Protopapa, E, Aggeli, A, Nelson, A, Boden, N, Salay, L, & Knowles, PF, Interaction of self-assembling beta-sheet peptides with phospholipid monolayers, *Medical Engineering & Physics*, in press.

Aggeli, A, Felton, S, Katta J & Ingham, E, Self-assembling peptides as injectable scaffolds for soft tissue engineering, *Nanomedicine*, in press

Carrick, LM, Aggeli, A, Ingham E, Fisher J, Effect of ionic strength on peptide self-assembly, *Tetrahedron Letters*, in press

Book chapters

Aggeli, A., Bell, M., Strong, A, Radford, S.E., & Boden, N., Self-assembling homopolymeric peptide tapes in aqueous solution, in *Peptide Science – Present and Future*, ed. Y.Shimonishi, Kluwer Acad. Press, 1999, pp.30-33.

Aggeli, A., Bell, M., Owens, R, Smith, A., & Boden, N., Self-assembling homopolymeric peptide tapes in moderately polar organic solvents, in *Peptide Science – Present and Future*, ed. Y.Shimonishi, Kluwer Acad. Press, 1999, pp.73-75.

Aggeli A, Nyrkova I, Bell, M, Carrick L, McLeish TCB, Semenov AN, Boden N, Exploiting peptide self-assembly to engineer novel biopolymers : tapes , ribbons, fibrils and fibres in *Self-assembling Peptide Systems in Biology Medicine and Engineering*, ed. N.Boden, A.Aggeli, S.Zhang, Kluwer Acad. Press, 2001, pp.1-17.

Aggeli, A, Bell, M, Boden, N, Carrick, L, Harding, R, McLeish, TCB, Nyrkova, IA, Semenov, AN, Impact of chirality on one-dimensional self-assembling systems, in *Self-Assembly*, edited by B.H. Robinson, IOS Press, 2003

McLeish, TCB, Mawer, P, Waigh, T, Aggeli, A, Boden, N, Semenov, AN, Nyrkova, I, Self-assembled peptide tapes : theory and experiment on self-assembly, kinetics and rheology, in *Mesoscale Phenomena in Fluid Systems*, ed. F.Case & P.Alexandridis, Oxford Press, 2004.

Aggeli, A, Bell, M, Boden, N, Carrick, L, Harding, R, McLeish, TCB, Nyrkova, IA, Semenov, AN, Impact of chirality on one-dimensional self-assembling systems, in *Chirality in Molecular Systems,* ed. B. Robinson, in press.

Aggeli, A, Boden, N, Carrick, L, McLeish, TCB, Nyrkova, IA, & Semenov, AN, Self-assembling peptide gels in *Molecular Gels*, editors P.Terech & R.G.Weiss, Kluwer Academic Press, 2005.

Other publications

Zhang, S, Boden, N, Aggeli, A, Self-assembly of peptides in medicine: two sides of the coin, ***Molecular Medicine Today***, 5, 512-513, 1999.

Aggeli, A., Radford, SE, & Boden, N., Exploiting protein folding and misfolding to engineer nanostructured materials, invited article, special issue on Nanotechnology, ***The Biochemist,*** 22, 10-14, 2000.

The Guardian, 20/3/1997

The Reporter, University of Leeds Staff Newsletter, 1/12/1997

Leeds Student, University of Leeds Student Newsletter, 23/1/1998

Book editor

Peptide self-assembly in biology, medicine and engineering, Crete, 1-6 July 1999, Kluwer Academic Press, eds N.Boden, S.Zhang and A.Aggeli, 2001

Scientific Advice :

Referee for EPSRC and for the Dutch Research National Council (2005-2006)

Member of the International Grants Panel, Royal Society (2006-2009)

Referee for *Biomacromolecules, JACS, Protein Science, Langmuir, Biochemistry, Soft Matter,*

Personal fellowships

Dorothy Hodgkin Royal Society Research Fellowship, 1997-2001

Royal Society University Research Fellowship, 2001-2009

EPSRC Advanced Research Fellowship, 2001- declined

Conference organization

Peptide self-assembly in biology, medicine and engineering, Crete, 1-6 July, 1999, organisers : N.Boden, S.Zhang, A.Aggeli

Peptide self-assembly in biology, medicine and engineering, Crete, 13-17 July 2001, organisers : S.Zhang, M.Hecht, N.Boden, A.Aggeli

Self-assembling fibrillar networks, ESF workshop, Crete July 2005, organizers: A.Aggeli, N.Boden, T.Waigh (Leeds), A.Donald (Cambridge)

TAB B

Peptide Primary structure	Macroscopic properties at 10mg.ml-1 in physiological solution conditions	Charge distribution pH = 7.5	Net charge pH=7.5	% β-sheet at 10mg.ml-1 in physiological – like solution
QQR$^+$FQWQFE$^-$QQ	Precipitate	+ -	0	85
QQQFQWQFE$^-$QQ	Precipitate	-	-1	85
QQR$^+$FE$^-$WE$^-$FE$^-$QQ	Viscous semi-transparent solution	+ - - -	-2	85
QQQFE$^-$WE$^-$FE$^-$QQ	Milky fluid		-3	65
QQE$^-$FE$^-$WE$^-$FE$^-$QQ	Clear fluid	- - - -	-4	55
E$^-$QE$^-$FE$^-$WE$^-$FE$^-$QE$^-$	Clear fluid	- - - - - -	-6	15
QQR$^+$FQWQFQQQ	Precipitate	+	+1	85
QQR$^+$FO$^+$WO$^+$FE$^-$QQ	Viscous semi-transparent solution	+ + + -	+2	65
QQ O$^+$F O$^+$W O$^+$FQQQ	Milky fluid	+ + +	+3	35
QQ O$^+$F O$^+$W O$^+$FO$^+$QQ	Clear fluid	+ + + +	+4	15

Table 1 : Amphiphilic self-assembling peptides with increasing negative or positive net charge in physiological solution conditions.

TAB C

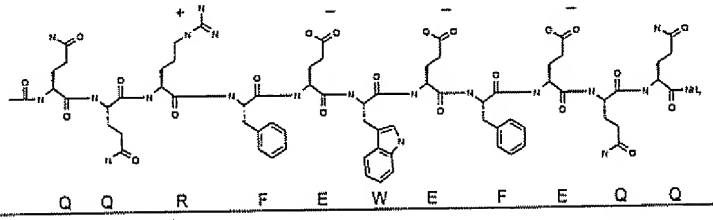
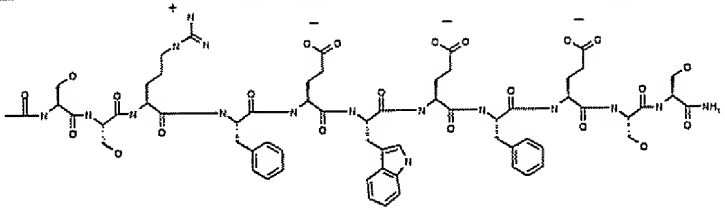
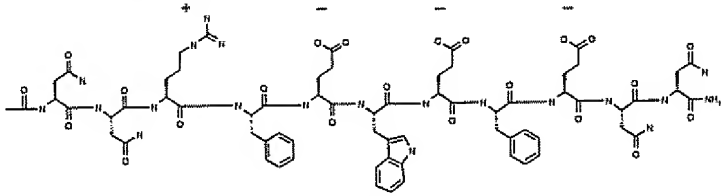
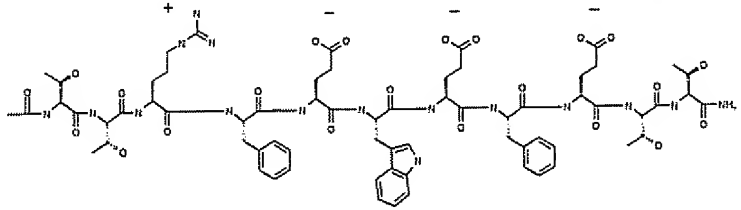
Net Charge at pH 7.5	Polar Amino Acid	Peptide Structure
-2	Glutamine	 Q Q R F E W E F E Q Q
-2	Serine	 S S R F E W E F E S S
-2	Asparagine	 N N R F E W E F E N N
-2	Threonine	 T T R F E W E F E T T

Table 2. Amphiphilic self-assembling peptides carrying a net negative charge in physiological solution.

TAB D

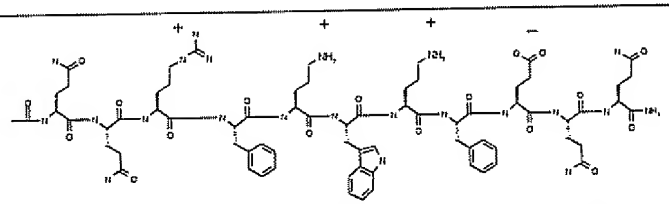
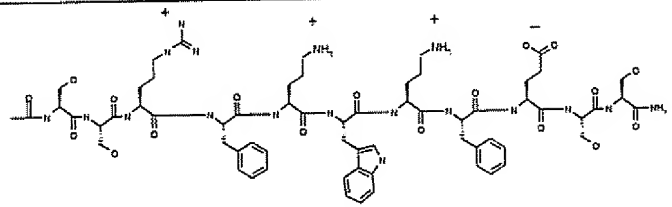
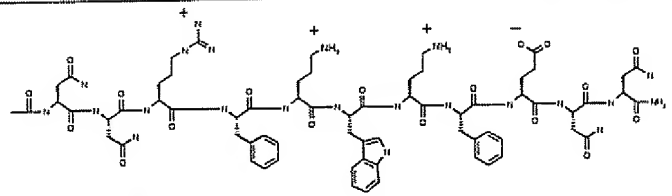
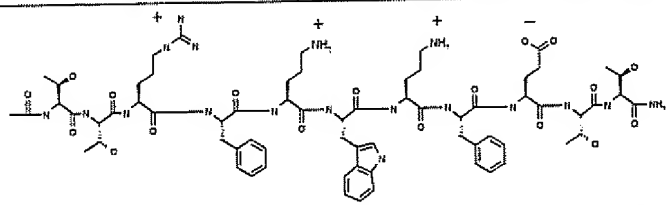
Net Charge at pH 7.5	Polar Amino Acid	Peptide Structure
+2	Glutamine	 Q Q R F O W O F E Q Q
+2	Serine	 S S R F O W O F E S S
+2	Asparagine	 N N R F O W O F E N N
+2	Threonine	 T T R F O W O F E T T

Table 3. Amphiphilic self-assembling peptides carrying a net positive charge in physiological solution.

TAB E

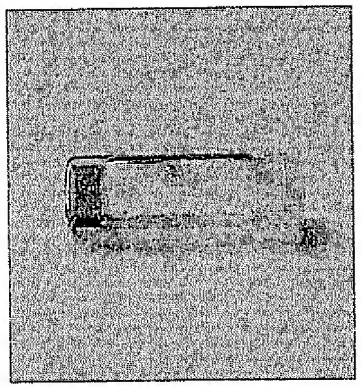


Figure 1. A self-supporting peptide gel in cell culture medium

TAB F

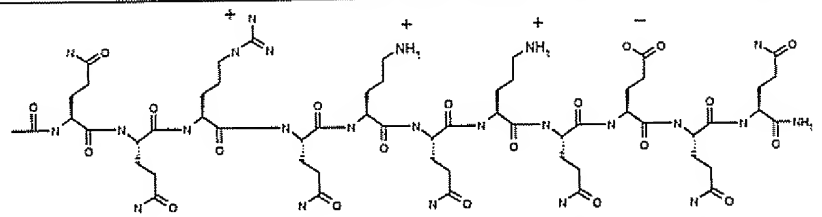
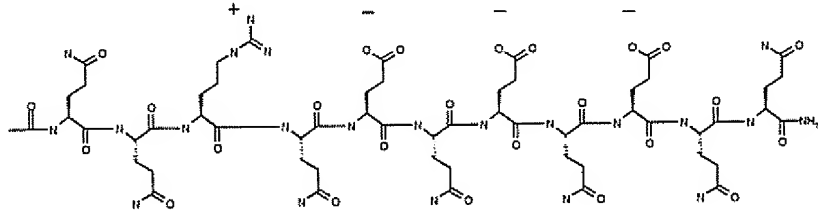
Net Charge at pH 7.5	Polar Amino Acid	Peptide Structure
+2	Glutamine	 Q Q R Q O Q O Q E Q Q
-2	Glutamine	 Q Q R Q E Q E Q E Q Q

Table 4. Polar self-assembling peptides in physiological solution.